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Manganese Hexacyanoferrate with Poly(3,4-ethylenedioxythiophene) Hybrid Film Modified Electrode for the Determination of Catechin and Melatonin

Tsung-Hsuan Tsai, Yun-Chin Huang , Shen-Ming Chen[*]

Electroanalysis and Bioelectrochemistry Lab,Department of Chemical Engineering and Biotechnology, National Taipei University of Technology,No.1, Section 3, Chung-Hsiao East Road, Taipei 106, Taiwan (ROC).
[*]E-mail: smchen78@ms15.hinet.net

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The manganese hexacyanoferrate (MnHCF) mixed-valent poly(3,4-Ethylenedioxythiophene) (PEDOT) hybrid film (MnHCF-PEDOT) was prepare on glassy carbon electrode by multiple scan cyclic voltammetry. These materials are characterized using atomic force microscopy (AFM), field emission scanning electron microscope (FE-SEM), energy dispersive spectroscopy (EDS), x-ray diffraction studies (XRD) and electrochemical impedance studies (EIS) techniques. The advantages of these films are demonstrated for detection of catechin and melatonin using cyclic voltammetry and amperometric. The electrocatalytic oxidation of catechin and melatonin at different electrode surfaces such as the bare GCE, MnHCF/GCE and MnHCF-PEDOT/GCE modified electrodes was explored in 0.1 M KCl solution at pH 1.5.The electrochemical sensor for catechin and melatonin exhibited a wide linear response range (0.1 to 4.4 M, R^2 = 0.9965 for catechin and 0.1 to 4.6 mM, R^2 = 0.9998 for melatonin), lower detection limit (0.01 mM) and fast response time (3 s).In addition, the MnHCF-PEDOT/GCE exhibited a distinct advantage of simple preparation, specificity, stability and reproducibility.

Keywords: Manganese hexacyanoferrate, Poly 3,4-Ethylenedioxythiophene, Electrocatalysis, Catechin, Melatonin

1. INTRODUCTION

Metal hexacyanoferrates show interesting redox chemistry that is accompanied by changes in their electrochromic, ion exchange, and electrocatalytic properties [1, 2]. Of particular interest in chemistry and materials science are electropolymerized polynuclear metal hexacyanoferrate films, and in particular, their use in modified electrodes.

Use of polynuclear metal hexacyanoferrates has led to the synthesis of conducting polymers [3, 4]. The polymer film-coated electrodes can be differentiated from other modification methods because of their adsorption and covalent bonding, in that they usually involve multilayers as opposed to the monolayers that are frequently encountered for the latter methods. Conductive/electroactive polymers, such as polypyrrole, polyaniline, polythiophene, etc., were prepared through an electropolymerization procedure and used as modifiers for the construction of chemically modified electrodes [5-8].

Among various conducting polymers, poly(3,4-ethylenedioxythiophene) (PEDOT), which is a relatively new and well-known p-conjugated conducting polymer of the polythiophene class, has received much attention because of its high electrical conductivity, moderate band gap, and excellent environmental stability [9-13]. Moreover, PEDOT thin films can vary from light to dark blue. These unique properties mean that PEDOT can be applied in many fields, such as chemical and biochemical sensors, antistatic coatings, electrically switchable windows, and polymer light-emitting diodes [14-16]. In recent reports, metal hexacyanoferrates (MHCFs) such as NdHCF [17], AgHCF [18], SnHCF [194], CoHCF [20], NiHCF [21], CuHCF [22], etc., MHCFs have also received much attention in many fields because of their special properties and potential applications [23-25].

Poly(3,4-ethylenedioxythiophene) (PEDOT) has been widely investigated as an electronically conducting polymer. It can be easily electrodeposited onto a surface by the electrooxidation of its monomer [26-29]. A PEDOT film in its oxidized form has been found to have high conductivity and stability at physiological pH [30, 31]. MnHCF can be prepared as an electroactive thin film of a Mn substrate in the presenceof ferricyanide anions [32, 33]. It is considered to be an attractive material for electrode surface modification owing to its well-defined, reversible, and reproducible responses in supporting electrolytes [34].

Catechin is a biomolecule that is widely present in numerous fruits and along with procycnadin-B3, chlorogenic acid, and caffeic acid is the major phenolic compound found in peaches [35, 36]. It also belonging to flavonoids has been acknowledged to be antioxidant and radical scavenger [37]. Applications of catchin as a phenolic standard in quantization of reducing equivalents in food and biological fluids and its oxidation mechanism are therefore of particular interest [38]. The interest in the quantification of catchin is consistent with its importance as potentially beneficial to human health.

The evidence of the presence of catechin in food stuffs and beverages can be related to epidemiological studies that take into account the relationships between catechin intake and diseases [39]. Its inhibition of lipid oxidation has been widely investigated and its beneficial action on cardiovascular diseases has been shown [40].

Melatonin is well known as a paracrine hormone that is secreted in a cyclic manner by the pineal gland [41]. In mammals, the pineal gland is believed to be the major source of circulating melatonin [42]. It detoxifies a variety of free radicals (OH), peroxynitrite anion, singlet oxygen, and nitric oxide [43]. Melatonin controls biological rhythms, pigment metabolism, immune response, metabolism of free radicals, monitoring of mood and sleep, cell proliferation and differentiation [44].

Traditional catechin analysis is mainly carried out by instrumental analysis, such as thin-layer chromatography (TLC) [45], capillary electrophoresis (CE) [46, 47], HPLC-UV [48]. However, such analysis is generally performed at centralized laboratories, requiring extensive labor and analytical

resources, and often results in a lengthy turnaround time [49]. Thus it is very important to establish a simple, fast, sensitive and low cost method for monitoring catechin. The electrochemical analysis has many advantages over conventional methods [50]. The sensitivity and selectivity of electrochemical analysis can be enhanced using chemically modified electrodes [53]. Hence, various electrochemical modified electrodes have been developed for the determination of catechin [51–53]. Moreover, in the previous works, the oxidation mechanisms of catechin have been explored [52, 54] via electrochemical methods.

All these methods could not reach a low enough detection limit. And the way to fabricate the modified electrodes was comparably complicated. Therefore, a low and simpler analytical method is urgently required. In this manuscript, we used MnHCF film that was immobilized on the PEDOT/GCE surface, and performed a voltammetric investigation on the redox behavior of catechin and melatonin in the aqueous media that provided valuable information in understanding the biological oxidation of catechin and melatonin.

2. EXPERIMENTAL

2.1. Materials

Manganese(II) sulfate were purchased from Wako (Japan). 3,4-ethylenedioxythiophene (EDOT) was purchased from Sigma-Aldrich (USA). Double distilled deionized (DDDI) water was used to prepare all solutions. The PEDOT film was prepared by electrochemical polymerization, using 0.1 M H_2SO_4 as supporting electrolyte. The buffer solution of MnHCF-PEDOT modified electrode tests was prepared using 0.1 M KCl as supporting electrolyte (pH 1.5). Pure nitrogen was passed through all the experimental solutions. All the chemicals used were of analytical grade.

2.2. Apparatus

All electrochemical experiments were performed using a CHI 750a potentiostat (CH Instruments, USA). The Bioanalytical Systems (BAS) glassy carbon electrode (GCE; diameter 0.3 cm, exposed geometric surface area 0.07 cm^2; Bioanalytical Systems, Inc., USA) was used. A conventional three-electrode system was used; it comprised a Ag/AgCl (saturated KCl) reference electrode, PEDOT/GCE, MnHCF/GCE and MnHCF-PEDOT/GCE modified electrodes, and a bare GCE electrode, as working electrodes, and platinum wire as counter electrode. Electrochemical impedance studies (EIS) were performed using a ZAHNER impedance analyzer (Germany). The atomic force microscope (AFM) images were recorded using a multimode scanning probe microscope (Being Nano-Instruments CSPM-4000, China). Field emission scanning electron microscope (FE-SEM) images were recorded using a HITACHI S-4700 (Japan). X-ray diffraction (XRD) experiments were carried out using an XPERT-PRO (PANalytical B.V., The Netherlands). Samples were scanned in the range 10–90° (2θ).

2.3. Preparation of Sample and Hybrid Film Modified Electrode

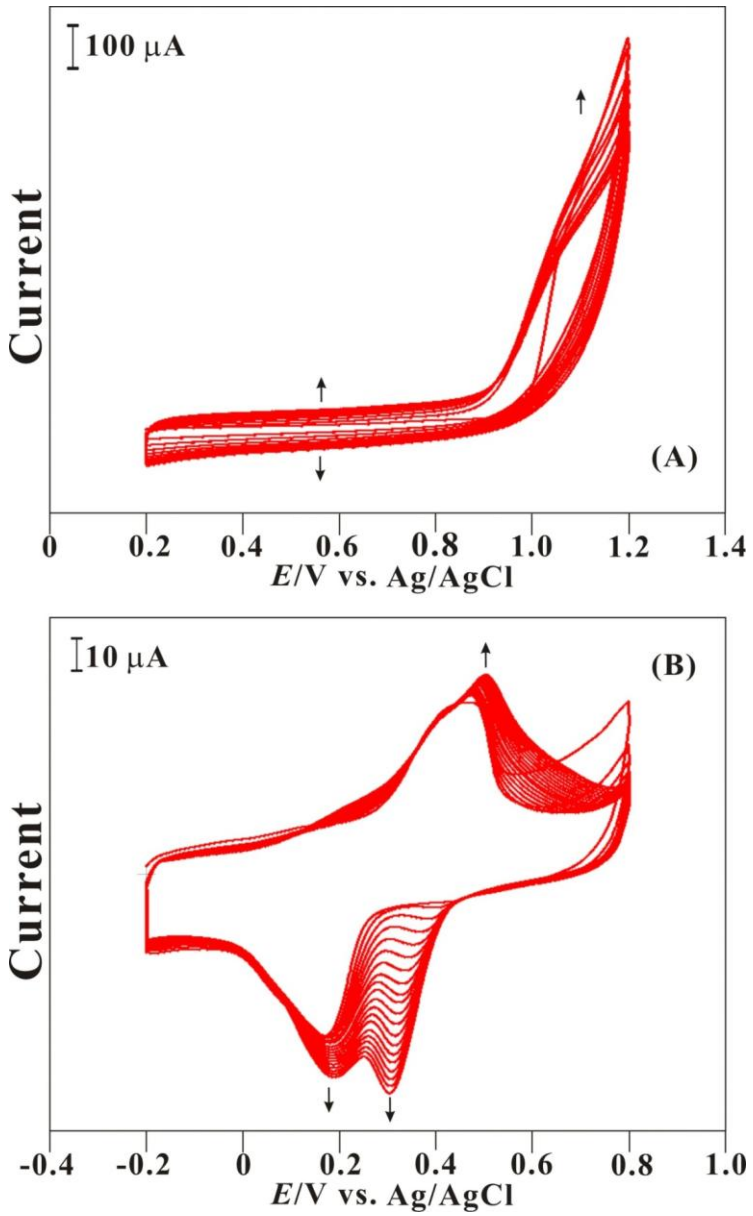

Figure 1. (A) CVs obtained by consecutive sweeps in 0.2 M $LiClO_4$ solution containing 0.01 M EDOT. Potential range +0.2 to +1.2 V. Scan rate 50 mV/s. **(B)** CVs of consecutive sweeps in 0.2 M $LiClO_4$ solution containing 1×10^{-3} M $K_3Fe(CN)_6$, 1×10^{-3} M $MnSO_4$, and 0.01 M EDOT. Potential range +0.2 to +0.8 V. Scan rate: 50 mV/s.

Prior to the electrochemical deposition process the GCE was well polished with aqueous slurries of alumina powder (0.05 μm), using a BAS polishing kit, then rinsed and ultrasonicated in DDDI water. Figure 1A shows the electrochemical polymerization of PEDOT film at GCE in 0.1 M $LiClO_4$ solution between +0.2 and +1.2 V, at the scan rate of 0.1 V/s for ten cycles. Figure 1B shows the electrochemical polymerization of the MnHCF film on PEDOT/GCE carried out by the cyclic voltammetric method in 0.1 M KCl solution containing 1×10^{-3} M $K_3Fe(CN)_6$ and 1×10^{-3} M $MnSO_4$.

The potential cycling was carried out within the potential range of -0.2 and +0.8 V, at the scan rate of 0.1 V/s for twenty cycles. The film formed in the aqueous 0.1 M KCl solution had three peaks at about +0.191, +0.312, and +0.517 V. The MnHCF-PEDOT/GCE was washed with deionized water and dried for 5 min. After polymerization, the electrode was treated with 0.1 M KCl (pH 1.5) solution by repeated cycling in the potential range –0.2 to +0.8 V, at the scan rate of 0.1 V/s, until a stable cyclic voltammogram (CV) was obtained.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Properties of MnHCF-PEDOT Hybrid Film Modified Electrode

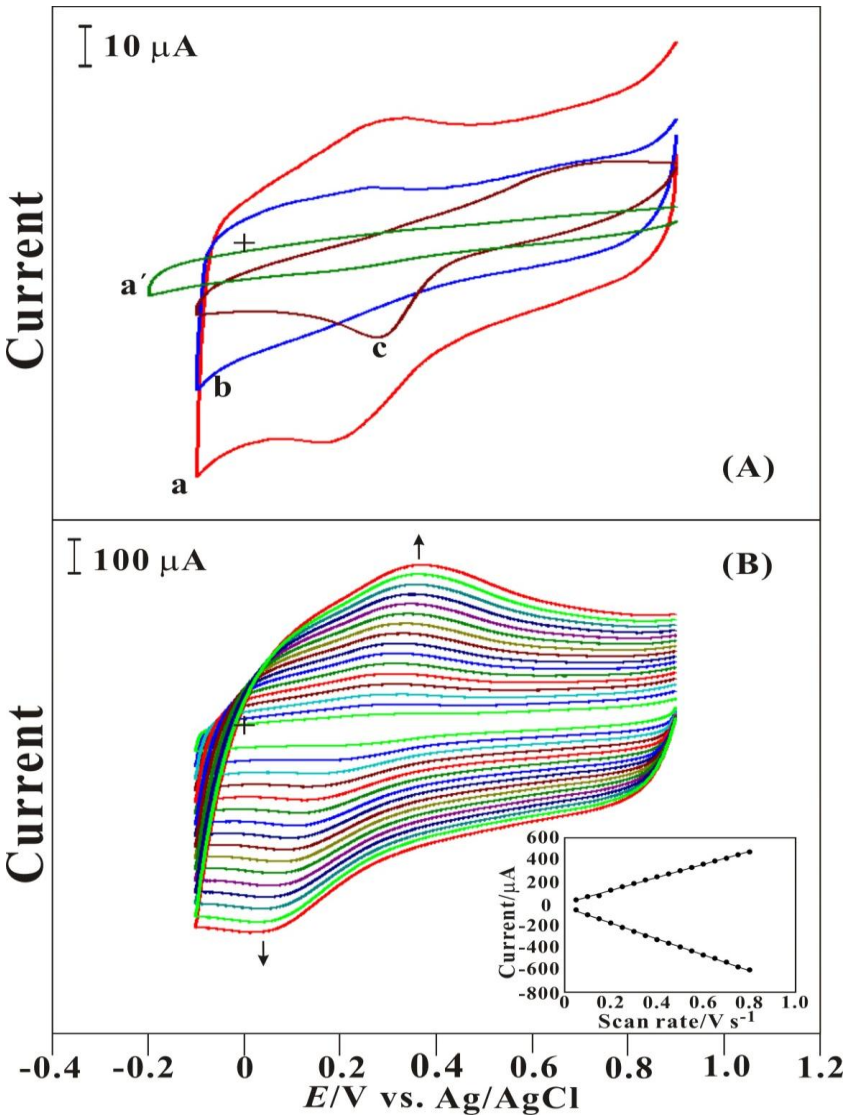

Figure 2. (A) CVs of (a) MnHCF-PEDOT, (b) PEDOT, (c) MnHCF film modified GCE and (a') bare GCE in 0.1 M KCl (pH 1.5). Scan rate = 50 mV/s. **(B)** Results of different scan rate studies of MnHCF-PEDOT modified GCE in 0.1 M KCl (pH 1.5). Scan rate in the range from 0.05 to 0.8 V/s.

The electrochemical properties of different modified electrode were investigated using CV (Figure 2A). In Figure 2A, curve (a) indicate the MnHCF-PEDOT/GCE, (b) PEDOT/GCE, (c) MnHCF/GCE and curve (a') bare GCE electrode CV signals. For the MnHCF/GCE modified electrode, the anodic and cathodic peak currents were significantly lower than observed for the MnHCF/PEDOT modified electrode.

This result might be the PEDOT film offers good stability, high conductivity, and acts as a good matrix. The MnHCF particles entrapped in the PEDOT film and helped the dispersion of MnHCF particles to improve the electrochemical signal. By the same way, there was no obvious response at curve (b) PEDOT/GCE and (a') bare GCE.

The MnHCF-PEDOT/GCE was used in several scan rate studies in 0.1 M KCl (pH 1.5). Figure 2B exhibits the different scan rate results of MnHCF-PEDOT/GCE in the range 0.05–0.8 V/s. As expected, the CVs of the MnHCF-PEDOT/GCE modified electrodes exhibited a single redox couple, with an anodic peak at +0.23 ~ +0.37 V and a cathodic peak at +0.19 ~ +0.06 V versus $Ag/AgCl/KCl_{sat}$. Here, the linear increase in the anodic and cathodic peak currents of MnHCF-PEDOT/GCE according to the scan rate revealed that the film exhibited the typical characteristics of surface controlled thin-layer electrochemical behavior. The inset (a) in Figure 2B shows the plot of the MnHCF-PEDOT signal of the anodic and cathodic peak current vs. scan rate. The corresponding linear regression equations were I_{pa} (μA) = 0.5881v (V/s) +1.826, R^2 = 0.9984, and I_{pc} (μA) = -0.7191v (V/s) – 31.912, R^2 = 0.9991. From Fig. 2A and B, the MnHCF combine with conducting polymer (PEDOT) seems to improve the redox peak current and more stable.

3.2. EIS and XRD Analyses

The electrochemical activity of the MnHCF-PEDOT/GCE was examined using the EIS technique. Here the complex impedance can be presented as a sum of the real Z' (ω) and imaginary Z'' (ω) components that originate mainly from the resistance and capacitance of the cell. From the shape of an impedance spectrum, the electron transfer kinetics and diffusion characteristics can be determined. The respective semicircle parameters correspond to the electron transfer resistance (R_{et}) and the double layer capacity (C_{dl}) nature of the modified electrode. As shown in Figure 3A, curve (a) indicates the Nyquist plot of MnHCF-PEDOT/GCE and (a') MnHCF/GCE in the presence of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) in KCl (pH 1.5) solution. The MnHCF-PEDOT/GCE shows a very small depressed semicircle arc with an interfacial resistance due to the electrostatic repulsion between the charged surface and probe molecule $Fe(CN)_6^{3-/4-}$. This depressed semicircle arc (R_{et} = 113 (Z'/Ω)) clearly indicates the lower electron transfer resistance behavior compared to that of the MnHCF/GCE (R_{et} = 168 (Z'/Ω)). These results clearly illustrate the electrochemical activities of the MnHCF-PEDOT and MnHCF films modified GCE, respectively.

As shown in Figure 3B, XRD analysis was used to determine the structure of the MnHCF-PEDOT film. The seven peaks that appear at around 43.5°(2θ) correspond to the (221) crystallographic plane, the peak at around 44.65°(2θ) corresponds to the (310) plane, the peak at around 48.84°(2θ) corresponds to the (311) plane,, the peak at around 50.62°(2θ) corresponds to the (222) plane, the peak

at around 64.93°(2θ) corresponds to the (331) plane, the peak at around 78.04°(2θ) corresponds to the (511) plane and the peak at around 87.99°(2θ) corresponds to the (441) plane (JCPDS 85-4253, 89-4086) [55, 56]. For PEDOT patterns, the peaks were found in 24.95°.For ITO patterns, the peaks were found in 38.41° and 74.42°. All these XRD peaks clearly validate the presence of MnHCF-PEDOT on the ITO surface.

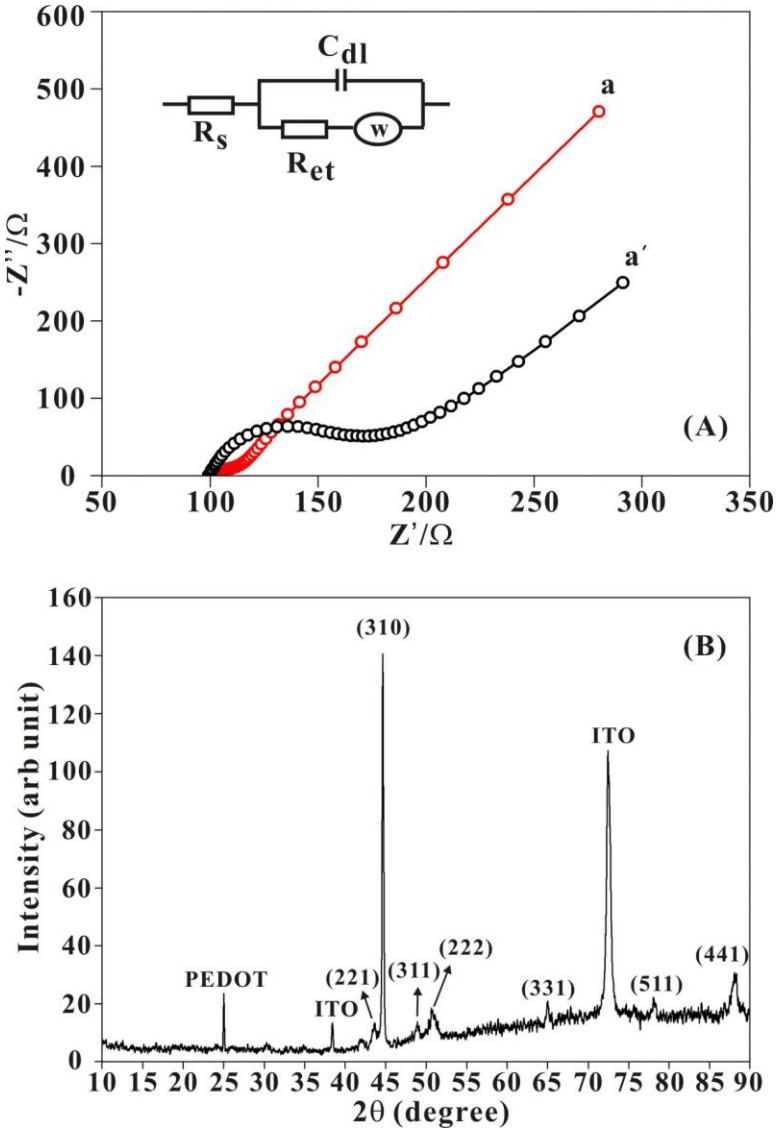

Figure 3. (A) Electrochemical impedance spectra curves of (a) MnHCF-PEDOT/GCE and (a') bare GCE in 0.1 M KCl (pH 1.5) solution containing 5×10^{-3} M $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1). **(B)** XRD spectra of a MnHCF-PEDOT film.

3.3. AFM and SEM Analyses

Scanning electron microscopy (SEM) and atomic force microscopy (AFM) were utilized to image the morphology of the active surface of the electrodeposited PEDOT, MnHCF and MnHCF-PEDOT films, as shown in Figure 4 (A)-(F). The SEM images show the (A) PEDOT film as a network

structure surface, (B) MnHCF film as a uniform nano particles with fiber dimension of 60 ~ 120 nm and (C) MnHCF-PEDOT film as MnHCF nano particles entrap PEDOT network structure film.

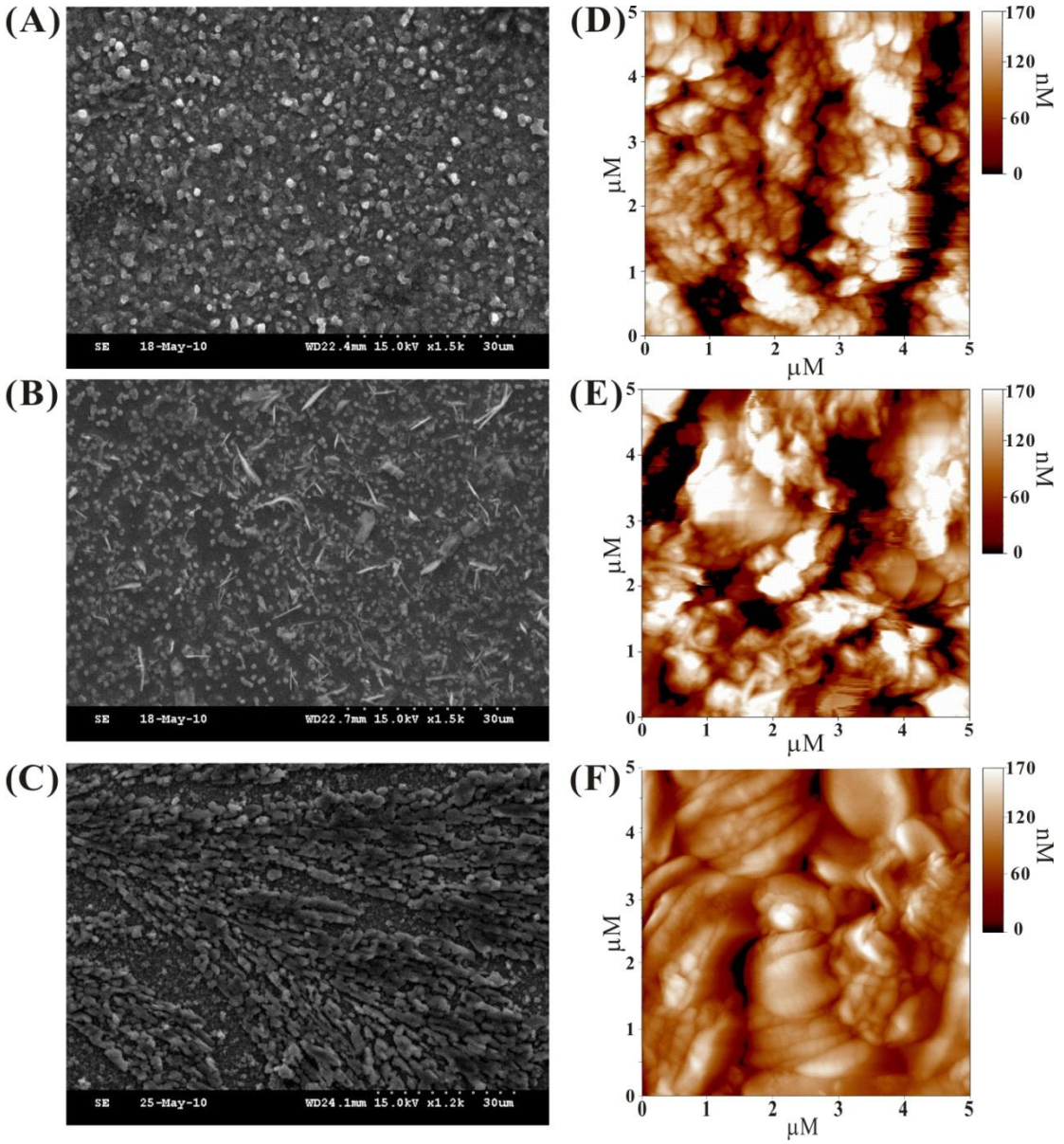

Figure 4. SEM images of (A) PEDOT/GCE, (B) MnHCF/GCE, and (C) MnHCF-PEDOT/GCE; and the tapping mode AFM images of (D) PEDOT/GCE, (E) MnHCF/GCE, and (F) MnHCF-PEDOT/GCE, respectively.

The AFM parameters have been evaluated for 5000 × 5000 nm surface area. The surface morphology of PEDOT, MnHCF and MnHCF-PEDOT films were examined by using the tapping mode. The AFM images of these films show the average diameter of 76.6 nm, 93.1 nm, and 79.2 nm the average height of 47.2 nm, 46.4 nm, and 34.5 nm. From the above results, the PEDOT network with a highly porous structure can be seen, which might easily entrap MnHCF nano particles.

3.4 Voltammograms of catechin oxidation at various electrode surfaces

3.4.1 Mediated Oxidation ofcatechin

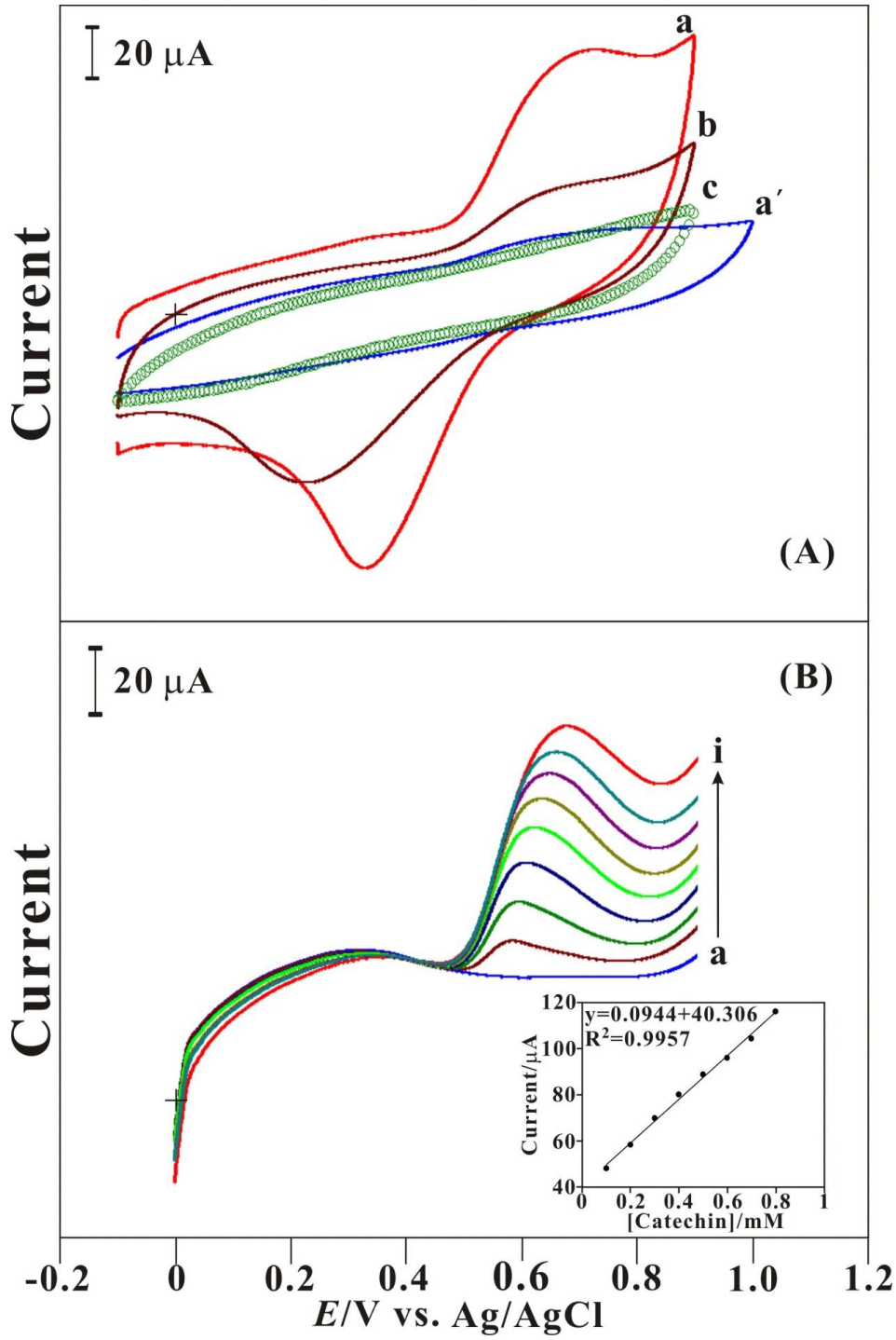

Figure 5. (A) Cyclic voltammetric responses at (a) MnHCF-PEDOT/GCE, (b) PEDOT/GCE, (c) MnHCF/GCE and (a') bare GCE in pH 1.5 KCl buffer solution containing $5x10^{-4}$ M catechin. Scan rate = 50 mV/s. **(B)** RDE voltammograms of MnHCF-PEODT film for the detection of catechin in pH 1.5 KCl buffer solution. Catechin concentrations were in the range of (a – i): 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8mM.Rotating speed: 1000 rpm. Inset: calibration plot of oxidation current vs. concentration of catechin.

Figure 5A depicts the cyclic voltammetric responses at (a) MnHCF-PEDOT/GCE, (b) PEDOT/GCE, (c) MnHCF/GCE and (a') bare GCE in pH 1.5 KCl buffer solution containing $5x10^{-4}$ M catechin. In the same solution, compare with PEDOT/GCE and MnHCF-PEDOT/GCE, the bare GCE and MnHCF/GCE show almost no response of catechin oxidation reaction. The PEDOT/GCE and the MnHCF-PEDOT/GCE have almost the same oxidation potential of catechin at +0.65 V, but the peak current of the PEDOT/GCE are much less than the MnHCF-PEDOT/GCE. All the above results indicate that using the MnHCF-PEDOT film modified electrode can help to enhance the electro catalytic reaction of catechin.

The RDE technique was employed for the detection of catechin in 0.1 M KCl buffer solution (pH 1.5). The rotation speed of MnHCF-PEDOT film modified GCE was set to be as 1000 rpm and the reduction progress has been examined within the potential of 0 to +0.9 V, scan rate = 0.1 V/s. Curve a–h of Fig. 5B show that there is a great increase in the cathodic peak current at MnHCF-PEDOT/GCE for the increasing concentrations of catechin (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mM) in 0.1 M KCl buffer solution (pH 1.5). The oxdiation peak current vs. concentration of catechin has been plotted and shown in the inset of Fig. 5B. The calibration plot is linear in the entire range (0.1 to 0.8 mM, R^2=0.9957) of catechin concentration studied with a sensitivity of 1.35 μA mM^{-1} cm^{-2}.These results clearly indicate the electrocatalytic catechin oxidation occurs at MnHCF-PEDOT film modified GCE.

3.4.2 Amperometric Response of Catechin Electrocatalysis by MnHCF-PEDOT Film

To study electrocatalytic oxidation of catechin by MnHCF-PEDOT film, MnHCF-PEDOT/GCE was applied in 0.1 M KCl buffer solution (pH 1.5) with the sequential additions of catechin by amperometry.

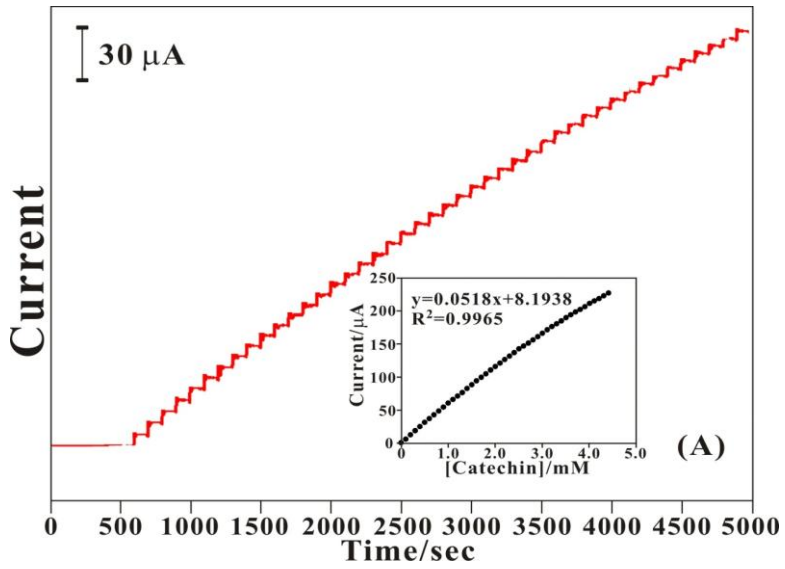

Figure 6. (A) Typical amperometric curve obtained for a MnHCF-PEDOT/GCE in 0.1 M KCl buffer solution (pH 1.5) at 0.65 V. Stirring rate 1000 rpm. Successive additions of catechin in the range from 0.1to 4.4mM (s/n = 3). Inset figure show the corresponding calibration plot with the concentration of catechin between 0.1 to 4.4 mM.

Fig. 6 show the amperometric responses of sequential additions of standard catechin (each 0.1 mM) tested by MnHCF-PEDOT/GCE in 0.1 M KCl buffer solution (pH 1.5), respectively, rotating speed = 1000 rpm, *Eapp.* = 0.65 V. During the period from 600^{th} to 5000^{th} second, it could be found almost linearly dependence between amperometric current and catechin concentration (shown in insets of Fig. 6) in both standard samples. For standard catechin detection (as shown Fig. 6), the sensitivity of MnHCF-PEDOT/GCE was 0.74 μA mM^{-1} cm^{-2} and the linear range spans the concentration of catechin from 0.1 to 4.4 mM with a correlation coefficient of 0.9965. The modified electrode was found has a lowest concentration detection limit of 0.01 mM with a 'signal-to-noise ratio' of 3. And it will be not linearly dependence with concentration as higher than 4.4 mM. Hence, 4.4 mM is suggested as the highest concentration detection limit for applying this film modified electrode in analytic grade catechin reagent.

3.5 Voltammograms of melatonin oxidation at various electrode surfaces

3.5.1 Mediated Oxidation of melatonin

The electrocatalytic oxidation of melatonin is studied and compared with different film modified electrodes in 0.1 M KCl buffer solution (pH 1.5) by voltammetry. Fig.7A shows the cyclic voltammograms of (a) MnHCF-PEDOT/GCE, (b) PEDOT/GCE, (c) MnHCF/GCE, and (a') bare GCE examined in 0.1 M KCl buffer solution (pH 1.5) containing $5x10^{-4}$ M melatonin, respectively. The proposed composites,(a) MnHCF-PEDOT/GCE, shows high electrocatalytic oxidation current for melatonin with a sharp cathodic peak as comparing to other electrodes at 0.41 V. By comparison, the MnHCF-PEDOT/GCE shows uniquely electrocatalytic ability of higher electrocatalytic current better than that of PEDOT/GCE, MnHCF/GCE and bare GCE. It represents MnHCF-PEDOT film has potential to develop melatonin sensor.

The RDE technique was employed for the detection of melatoninin 0.1 M KCl buffer solution (pH 1.5) in Fig. 7B. The rotation speed of MnHCF-PEDOT film modified GCE was set to be as 1000 rpm and the oxidation progress has been examined within the potential of 0 to +0.9 V, scan rate = 0.1 V/s. Curve a–j of Fig. 7B show that there is a gradual increase in the oxidation peak current at MnHCF-PEDOT/GCE for the increasing concentrations of melatonin (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 mM) in 0.1 M KCl buffer solution (pH 1.5). The oxidation peak current vs. concentration of melatonin has been plotted and shown in the inset of Fig. 7B. The calibration plot is linear in the entire range (0.5 to 4.5 mM, R^2=0.9948, n=3) of melatonin concentration studied with a sensitivity of 4.9 μA mM^{-1} cm^{-2}. The detection limit was found to be 0.1 mM.

3.5.2 Amperometric Response of Melatonin Electrocatalysis by MnHCF-PEDOT Film

The amperometric response of the MnHCF-PEDOT hybrid film modified electrode to melatonin was investigated in a stirred 0.1 M KCl buffer solution (pH 1.5) at a working potential of +0.41 V. Figure 8 shows typical current–time curves for successive additions of melatonin for different concentrations, between 0.1 and 4.6 mM, with the MnHCF-PEDOT hybrid film modified electrode.

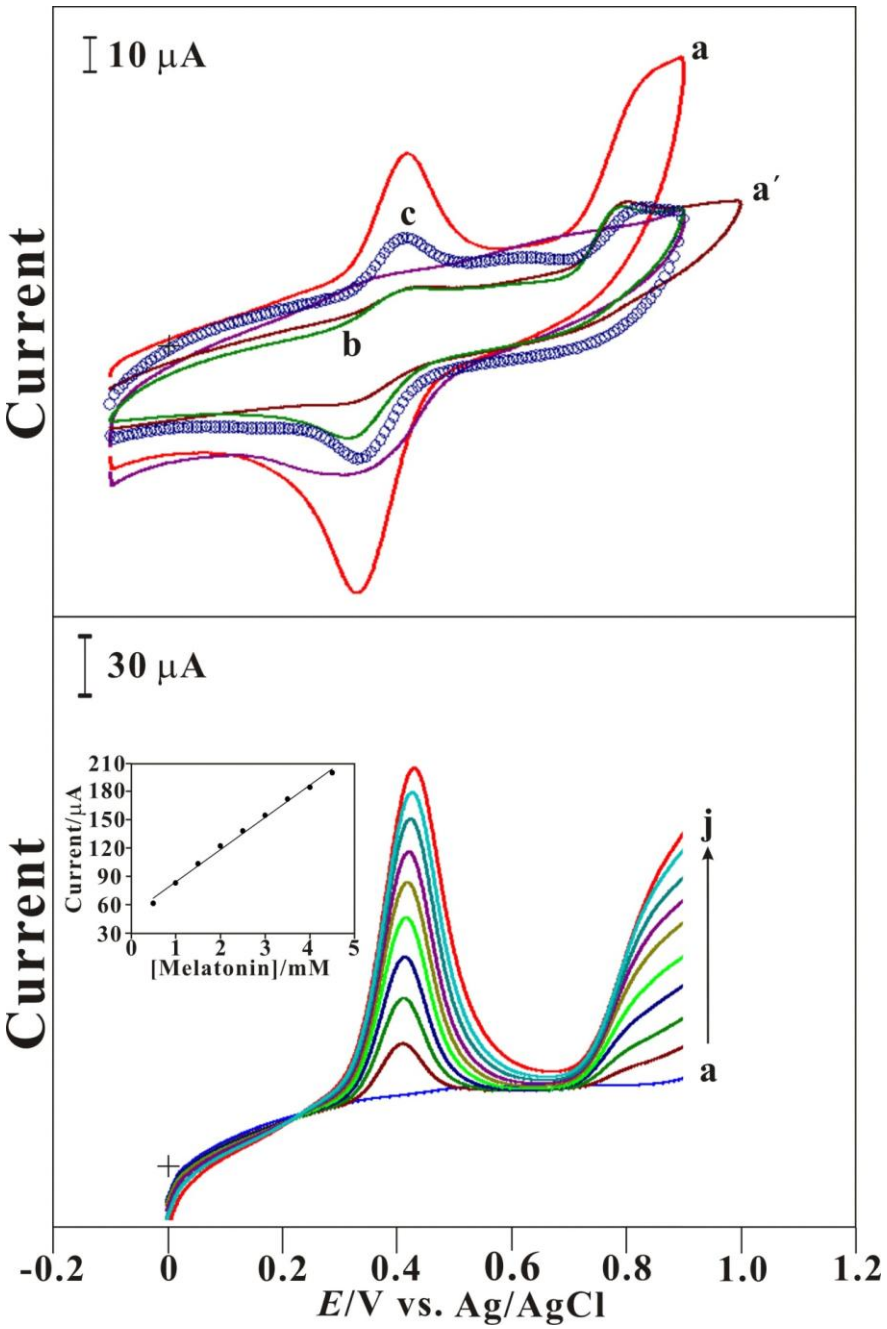

Figure 7. (A) Cyclic voltammetric responses at (a) MnHCF-PEDOT/GCE, (b) PEDOT/GCE, (c) MnHCF/GCE and (a') bare GCE in pH 1.5 KCl buffer solution containing $5x10^{-4}$ M melatonin. Scan rate = 50 mV/s. **(B)** RDE voltammograms of MnHCF-PEODT film for the detection of melatonin in pH 1.5 KCl buffer solution. Melatonin concentrations were in the range of (a – j): 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 mM. Rotating speed: 1000 rpm. Inset: calibration plot of oxidation current vs. concentration of melatonin.

The current immediately changed after the addition of melatonin and reached another steady-state current within 3 s. The inset of Figure 8 shows the calibration curve of the MnHCF-PEDOT hybrid film modified electrode with the concentration of melatonin between 0.1 to 4.6 mM (the correlation coefficient were 0.9998). Thus the present modified electrode shows a good linear response

for the electrocatalytic oxidation of melatonin in the range 0.1 to 4.6 mM and detection limit was 0.01 mM (s/n = 3).

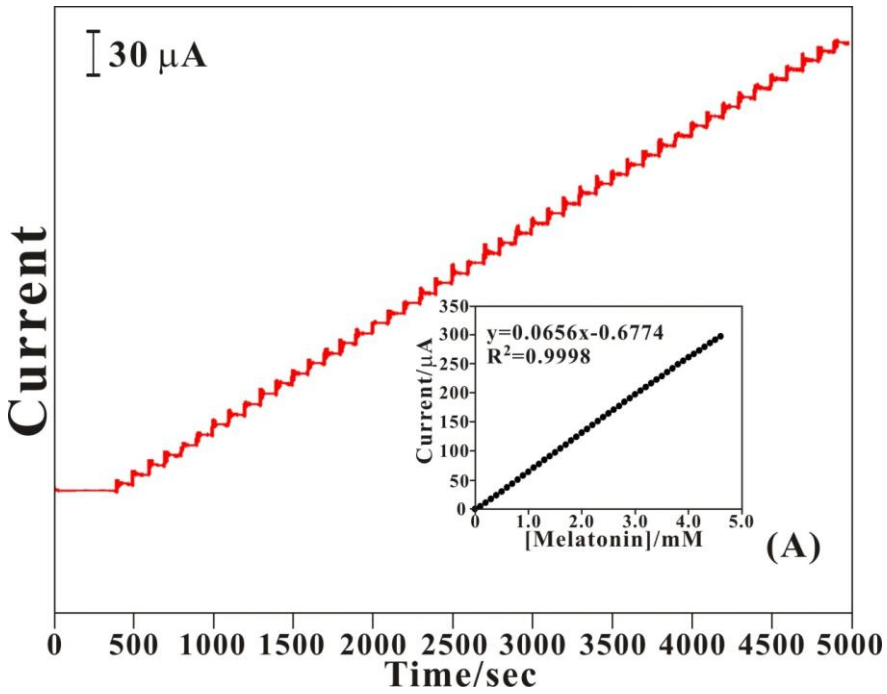

Figure 8. (A) Typical amperometric curve obtained for a MnHCF-PEDOT/GCE in 0.1 M KCl buffer solution (pH 1.5) at 0.41 V. Stirring rate 1000 rpm. Successive additions of melatonin in the range from 0.1 to 4.6 mM (s/n = 3). Inset figure show the corresponding calibration plot with the concentration of melatonin between 0.1 to 4.6 mM.

The linear concentration range, sensitivity and limit of detection observed with MnHCF-PEDOT/GCE are in general comparable with most of the modified electrode reported in the literature [52-56] (Table 1). The MnHCF-PEDOT/GCE modified electrode for the detection of catechin and melatonin also has wide linear concentration range, lower detection limit and higher sensitivity.

3.6 Stability and Reproducibility

The reproducibility of the current response of the biosensor was examined by measuring catechin and melatonin concentration both of 5 x 10^{-4} M, and the relative standard deviation was 2.87 (*n* =5). It was indicated that the biosensor possessed good reproducibility. In addition, the catalytic current response for oxidation of melatonin at MnHCF-PEDOT/GCE was tested in 0.1 M KCl buffer solution (pH 1.5) containing 5 x 10^{-4} M melatonin before and after continuously stirring the buffer solution for 30 min. The response of the electrode signal had no significant change before and after stirring the solution; this test indicated that reproducible results can be obtained at the MnHCF-PEDOT/GCE. The stability of MnHCF-PEDOT hybrid film modified electrode was then investigated by storing it at room temperature in the presence of 0.1 M KCl buffer solution (pH 1.5).

Table 1. Comparison of the determination of catechin and melatonin by various electrochemical modified electrodes. Linear concentration range (LCR). Limit of detection (LOD).

Modified electrode	Analyte	E_{app} (mV)	Electrolyte	LCR (μM)	LOD (μM)	Sensitivity (μA mM^{-1} cm^{-2})	Reference
HP-β-CD/CPE	Catechin	+540 (---)	0.1M BRB[(a)] (pH 4.4)	0.34-24.11	0.001	---	[57]
SWCNT-CTAB/GCE	Catechin	+200 (Ag/AgCl, 3M KCl)	PBS (pH 7.0)	0.000372-0.0024	0.000112	---	[58]
Ni(II) complex–SAM/AE[(b)]	Catechin	+180 (Ag/AgCl, 3M KCl)	PBS (pH 7.0)	3.31-25.3	0.826	---	[59]
poly(fuchsin acid)/GCE	Melatonin	+730 (Ag/AgCl, sat. KCl)	0.1 M H_2SO_4 (pH 1.5)	50-200	---	---	[60]
Carbon fiber/ME[(c)]	Melatonin	+850 (Ag/AgCl, sat. KCl)	0.01 M MES[(d)] (pH 5.44)	0.39-32.5	0.0013	0.0042	[61]
MnHCF-PEDOT/GCE	Catechin Melatonin	+650 +410 (Ag/AgCl, sat. KCl)	0.1 M KCl (pH 1.5)	100-4400 100-4600	10 100	1.35 4.9	This work

(a) Britton-Robinson buffer
(b) Au electrode
(c) Microdisk electrode
(d) 2-(*N*-morpholinol)-ethanesulfonic acid

It was stable for one month but thereafter there was a gradual decrease (12%) in the current values. When the MnHCF-PEDOT hybrid film modified electrode store for a week in 0.1 M KCl buffer solution (pH 1.5), the voltammetric response current of catechin and melatonin decreased by 10% of the initial current. These results suggest that the MnHCF-PEDOT/GCE has high stability and good reproducibility.

4. CONCLUSIONS

A MnHCF-PEDOT hybrid film was successfully electro deposition on GCE. The MnHCF-PEDOT hybrid film was characterized using AFM, FE-SEM, EIS, and XRD. The XRD peaks clearly validate the presence of MnHCF-PEDOT on the ITO surface. EIS results help to conclude PEDOT can help not only inert materials to modify on the electrode surface but also reduce R_{et} (electron transfer resistance) of system. The MnHCF-PEDOT hybrid film modified electrode displays a linear response in the range of 0.1 to 4.4 mM catechin and 0.1 to 4.6 mM melatonin, with a correlation coefficient of

0.9965 and 0.9998. The detection limit was both found to be 0.01 mM and the response time was 3 s. This new method can be applied for the electroanalysis of catechin and melatonin.

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